Gut Microbes as Participants and Targets in Cardiometabolic Diseases

Stanley L Hazen, MD, PhD
Section Head, Preventive Cardiology, Cleveland Clinic
Chair, Dept. of Cellular & Molecular Medicine, Lerner Research Institute

Disclosures: all research presented was funded by the NIH and the Office of Dietary Supplements

- Dr. Hazen is named as a co-inventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and/or therapeutics.

- Dr. Hazen reports having been paid as a consultant for the following companies: Esperion, and Procter & Gamble.

- Dr. Hazen reports receiving research funds or support from Abbott, Astra Zeneca, Pfizer, Procter & Gamble, Roche, and Takeda.

- Dr. Hazen reports having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics and/or therapeutics from Cleveland Heart Laboratory, Esperion, Frantz Biomarkers, LLC and Siemens.

- Dr. Hazen was the scientific founder of Cleveland Heart Laboratory, and reports having equity in that company.

Gut Microbes
- break down toxins
- make vitamins and essential amino acids
- promote intestinal health/immunity
- create a barrier against invaders

There are ~10^{23} microbes in the gut; they make up 30-50% of feces (dry weight)

An estimated 3.3 million different genes are present amongst the intestinal microbial community.

This vastly outnumbers the 23,000 or so genes in the human genome

Homo sapien DNA constitutes only ~10% of the total DNA in each of us

"Obese microbiome" has an increased capacity for dietary energy harvest, and is a transmissible trait

Diet and Intestinal Microbes are Mechanistically Linked to Atherosclerotic Heart Disease

Meta-organisinal pathway:

(i) gut microbiota
(ii) host hepatic FMOs

Trimethylamine (TMA)
RA Koeth (2013) Nature Medicine
B Bennett (2013) Cell Metab
Z Wang (2014) Eur Heart J
WHW Tang (2014) JACC
RA Koeth (2014) Cell Metab
C Organ (2016) Circ Heart Fail

Strategy of metabolomics study design for identifying unbiased small molecule profiles predictive of incident risks for major adverse cardiovascular events

GeneBank (N=10,000)

Learning Cohort
50 cases (3yr MI, CVA, death) vs 50 age/gender matched controls

Validation Cohort
25 cases (3yr MI, CVA, death) vs 25 age/gender matched controls

Choline, betaine and trimethylamine-N-oxide are plasma analytes associated with CVD

Additional take home concepts:

- The microbiome is a filter of our largest environmental exposure - what we eat
- The microbiome can be considered as our largest endocrine organ
- The microbiome is a "drugable" target

Choline, betaine and trimethylamine-N-oxide (TMAO) are plasma analytes associated with CVD

Identities confirmed by:
LC-MS, 1H, 13C, 15N NMR
GC/MS/MS, Isotope tracer studies
Plasma levels of three phosphatidylcholine metabolites, choline, TMAO and betaine, predict CVD risks (N=1865)

Prospective Cohort: Sequential Cardiology Patients

Intestinal Microbial Organisms Play an Obligatory Role in TMAO Generation from Dietary Egg Yolk PC in Mice

Plasma levels of the gut flora dependent metabolite TMAO predict incident (3 year) CVD risks

New Independent Cohort: N=4007 Sequential Subjects

TMAO is a gut flora dependent metabolite in humans:

PC challenge - Oral d9-PC and 2 hard boiled eggs at each visit

Adjusted for age, sex, DM, HTN, smoking, LDL, HDL, TG, CRP, eGFR


WHW Tang et al, NEJM (2013)
Dietary choline induces atherosclerosis in the presence of intact gut flora (TMAO formation)

Intact flora
Abx

TMAO
apoE-ko, C57Blk6/J mice

Carnitine, an abundant nutrient in red meat, is also pro-atherogenic

Vegetarians/Vegans have lower synthetic capacity than Omnivores to form TMAO from carnitine because of altered gut microbial composition

TMAO alters macrophage phenotype, and sterol metabolism in multiple compartments


What are Dietary Sources of Choline/Phosphatidylcholine?

Lekithos (Greek) = Egg yolk
Chole (Greek) = Bile

Adherence to Mediterranean Diet Lowers Urinary TMAO Levels

De Filippis et al, Gut 2015

Choline TMA Lyase (CutC/D)
Carnitine TMA Lyase (Cnt A/B)
Promiscuous TMA Lyase (yeaX/W)

Promiscuous TMA Lyase (yeaX/W)

Screens
- primary
- secondary
- tertiary
- human commensals

Sources of DMB
- Olives/Cold-pressed extra virgin olive oil
- Grape seed oil
- Guinness Lager Stout

Dimethylbutanol (DMB) inhibits TMA production from multiple nutrients in human fecal commensals

Development of inhibitors (and activators) of microbial TMA lyases

Wang et al, Cell (2015)
DMB is a non-lethal microbial TMA lyase inhibitor in multiple human commensals.

New concept: Non-lethal microbial enzyme targeting as a therapeutic small molecule inhibition of microbial choline TMA-lyase activity.

Microbial TMAO lyase inhibition attenuates dietary choline enhanced atherosclerosis.

Microbial TMA-lyase inhibition reduces TMAO levels in vivo.

Drugging the Microbiome - Its in Our Future for CVD Therapeutics.

Wang et al, Cell (2015)
Drugs for Bugs
Drugging the Microbiome